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EFFECTS OF PHARMACOLOGIC INTERVENTION ON OXYGENATION, LUNG WATER AND
PROTEIN LEAK IN THE PSEUDOMONAS ARDS PORCINE MODEL

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Annual Report

July 1, 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-86-C-6168

Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia 23298-0519

DOD DISTRIBUTION STATEMENT

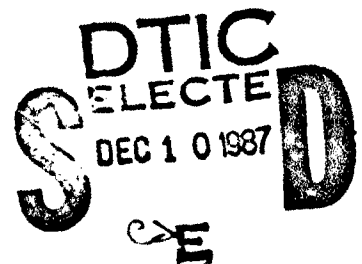
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20030128253

87 11 27 245

AD-A189 309



REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

| | | | |
|---|---|---|----------------------------------|
| 1a. REPORT SECURITY CLASSIFICATION Unclassified | | 1b. RESTRICTIVE MARKINGS A189307 | |
| 2a. SECURITY CLASSIFICATION AUTHORITY | | 3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release: distribution unlimited | |
| 2b. DECLASSIFICATION/DOWNGRADING SCHEDULE | | | |
| 4. PERFORMING ORGANIZATION REPORT NUMBER(S) | | 5. MONITORING ORGANIZATION REPORT NUMBER(S) | |
| 6a. NAME OF PERFORMING ORGANIZATION Medical College of Virginia | 6b. OFFICE SYMBOL (if applicable) | 7a. NAME OF MONITORING ORGANIZATION | |
| 6c. ADDRESS (City, State, and ZIP Code) Virginia Commonwealth University Richmond, Virginia 23298-0519 | | 7b. ADDRESS (City, State, and ZIP Code) | |
| 8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command | 8b. OFFICE SYMBOL (if applicable) | 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-86-C-6168 | |
| 8c. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, MD 21701-5012 | | 10. SOURCE OF FUNDING NUMBERS | |
| | | PROGRAM ELEMENT NO. 62772A | PROJECT NO. 3S162. 772A874 |
| | | TASK NO. AE | WORK UNIT 160 |
| 11. TITLE (Include Security Classification) Effects of Pharmacologic Intervention on Oxygenation, Lung Water and Protein Leak in the Pseudomonas ARDS Porcine Model | | | |
| 12. PERSONAL AUTHOR(S) Sugerman, H.J., Byrne, K., Tatum, J.L., and Hirsch, J. | | | |
| 13a. TYPE OF REPORT Annual Report | 13b. TIME COVERED FROM 7/1/86 TO 6/30/87 | 14. DATE OF REPORT (Year, Month, Day) 1987 July 1 | 15. PAGE COUNT |
| 16. SUPPLEMENTARY NOTATION | | | |
| 17. COSATI CODES | | 18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) | |
| FIELD | GROUP | SUB-GROUP | |
| 06 | 05 | | |
| 06 | 16 | | |
| 19. ABSTRACT (Continue on reverse if necessary and identify by block number) | | | |
| 20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS | | 21. ABSTRACT SECURITY CLASSIFICATION Unclassified | |
| 22a. NAME OF RESPONSIBLE INDIVIDUAL Judy Pawlus | | 22b. TELEPHONE (Include Area Code) 301/663-7325 | 22c. OFFICE SYMBOL SGRD-RMT-S |

SUMMARY

The adult respiratory distress syndrome is a condition which occurs as a result of both direct and indirect pulmonary injury. The mortality rate for the syndrome which may affect previously fit patients is over 50% and higher where sepsis predominates. This mortality, despite modern techniques in intensive care, has hardly changed in 20 years. The pathophysiological changes in the condition result in respiratory failure requiring endotracheal intubation and mechanical ventilation, the appearance of diffuse fluffy infiltrates on chest roentgenogram, a normal pulmonary wedge pressure, an arterial:alveolar pO_2 ratio ≤ 0.2 , and a total static lung compliance of ≤ 50 ml H_2O .

100-100
from 7 → At the cellular level, the lung injury is due to damage of the alveolar capillary membrane by various circulating elements of the blood, most notably neutrophils. These neutrophils become "activated" when they come in contact with a soluble or phagocytic stimulus, break down and release many inflammatory mediators. Among these mediators are the products of the cyclooxygenase and lipoxygenase systems of arachadonic acid breakdown such as the prostaglandins, leukotrienes, SRS-A, the complement factors C5a, C3a and oxygen-free radicals.

All of these substances are toxic to the alveolar capillary membrane and eventually cause its disintegration with concomitant protein leak across the damaged membrane into the lung. When the lymphatic clearance capacity of the lung is exceeded, pulmonary edema occurs and the clinical picture seen in ARDS unfolds.

Measurements of the degree of protein flux across the lung membrane is paramount in both the diagnosis and treatment of ARDS. Techniques developed in this laboratory using human serum albumin with a 99^m technetium label and

measured with a gamma camera over the right lung and heart, and expressed as the lung-heart ratio have been used successfully in the diagnosis of ARDS in both experimental and clinical models and have been used to great effect in elucidation of the efficacy of various pharmacological agents in the attenuation of the changes seen in the syndrome. Pseudomonas-induced ARDS in the porcine model has been used as a representative example of the syndrome in this laboratory.

2 Because ARDS is mediated by numerous inflammatory mediators, it is likely that treatment will require several pharmacological blocking agents. We have already established that treatment with cimetidine, diphenhydramine, H_2 and H_1 blockers, respectively, and ibuprofen, a prostaglandin antagonist, given i.v. at 20 and 120 minutes after pseudomonas infusion, significantly attenuates both the early hypertensive and late permeability phases of the syndrome as measured by hemodynamic parameters, blood gases and slope index (the ratio of change of radioactivity between the heart and right lung) and measurement of the extravascular lung water by the indicator dilution technique.

Delayed treatment in the model after 90 minutes of intravenous Pseudomonas has been shown to significantly increase survival time. Deleterious effects of ibuprofen on renal blood flow may well be improved by the concomitant administration of cimetidine and diphenhydramine and these data are at present undergoing analysis. Because of associated mental status changes in patients on long-term cimetidine in the intensive care situation, ranitidine, a H_2 blocker without these side effects, has been shown to be as efficacious in the treatment of experimental ARDS as cimetidine. Addition of heparin to CID did not improve any parameters over CID alone.

An anti-platelet activating factor SRI 63-675 has been shown to attenuate the early phase of pulmonary hypertension in the model but caused severe hemolysis, thus making it impractical at this time.

Depends. Blood platelets, leukocytes, monocytes, macrophages, prostaglandins, leukotrienes

Further studies on the effect of oxygen-free radicals in the model and the use of free radical scavengers are at present ongoing.

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| Availability Codes | | |
| Dist | Avail and/or Special | |
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FOREWORD

In conducting the research described in this report, the investigators adhere to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. NIH 78-23, Rev. 1978).

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PROBLEM

→ The adult respiratory distress syndrome (ARDS), as first described by Ashbaugh (1) 20 years ago, is a pathophysiological pulmonary condition of multiple etiologies. The syndrome may be initiated by direct pulmonary injury or may be seen as the lungs' response to a remote or systemic insult. Recognition that certain direct pulmonary and non-pulmonary conditions are responsible for ARDS has not altered the case fatality rate, which is about 50% or even higher when sepsis predominated (2). In civilian life the most common causes of ARDS are multiple trauma, aspiration of gastric contents, sepsis and pancreatitis. In combat soldiers, the condition known as the traumatic wet lung syndrome during the Korean conflict and Da-Nang lung in the Viet Nam war, and now recognized as ARDS, results from blast injuries, direct lung contusion, burn inhalation, inhalation of toxic substances, aspiration, multiple transfusions and as a complication of sepsis. As such, the condition often affects previously fit and healthy patients with a considerable mortality. → pg 2

Clinically, patients are considered to have the syndrome when certain criteria are met: respiratory failure requiring endotracheal intubation and mechanical ventilation, the appearance of diffuse pulmonary infiltrates on chest roentgenogram, an initial pulmonary wedge pressure of 18 mmHg or less, an arterial to alveolar PO_2 ratio ≤ 0.2 and a total static lung compliance of ≤ 50 ml H_2O .

Applying these criteria, between 150,000 and 200,000 patients in the United States are affected annually by the syndrome. The main stay of treatment is supportive therapy, treatment of the underlying disorder, the maintenance of adequate oxygenation with mechanical ventilation and routine end-expiratory pressure (PEEP), fluid balance, nutrition and antibiotics where indicated, since patients with ARDS are more susceptible to nosocomial infections.

BACKGROUND

Despite the multiple causes of ARDS, the final pathways end in the same result, i.e., damage to the pulmonary capillary membrane with increased permeability with accumulation of water and protein rich fluid in the pulmonary interstitium. Alveolar flooding occurs when interstitial and lymphatic clearance capacity are exceeded, leading to the perfusion of unventilated alveoli manifested as hypoxemia which is refractory to increased inspired oxygen concentrations.

It is likely that successful treatment of ARDS will involve intervention to prevent capillary endothelial damage and protein leak across the membrane. Consequently, accurate estimation of the onset and degree of protein flux are of paramount importance in clinical and experimental ARDS if treatment regimens are to be adequately assessed. These techniques should be sensitive and specific for the syndrome, and should be as safe and noninvasive as possible. In addition, they should be portable and give online results (3).

The measurement of extravascular lung water by indicator dilution techniques first described by Chinnard and Enns in 1954 (4) fits most of the above criteria and withstands the test of time. The use of radioisotopically tagged tracer proteins has provided an important technique for the estimation of lung leak in recent years. In this laboratory the use of radiolabeled ^{99m}technetium human serum albumin as a tracer protein across the damaged alveolar capillary membrane has been used in dogs given intravenous oleic acid (5) and pigs given intravenous live *Pseudomonas*.

Radioactive TcHSA distributes within the whole body pool after intravenous injection and remains essentially within the vascular compartment (6). Its distribution within the body can be imaged with a gamma camera. Using the

computerized gamma camera, data are collected at one-second intervals for 60 seconds and then at one-minute intervals for the duration of the study. During the initial pass of the radiopharmaceutical, it is possible to define the lungs and the heart anatomically for subsequent computer analysis and construction of lung:heart radioactivity ratios. This ratio remains constant unless a pulmonary microvascular membrane injury is present when a rising ratio termed "slope injury" (SI) is present. We have found in previous studies that the SI was proportional to the severity of injury and was more sensitive than either arterial blood gas analysis or standard chest roentgenograms (7). We have found that the leak of TcHSA was much greater than the leak of 99^m tagged RBC's (8) that PEEP did not alter the rate of pulmonary protein leak (9), that altered pulmonary vascular recruitment did not produce a rising radioactivity ratio following hemodynamic equilibration (10), and that multiple doses of TcHSA were associated with reproducible SI's over six hours following oleic acid administration in dogs. The method has been used in clinical trials with success to differentiate between cardiogenic and noncardiogenic pulmonary edema (11).

This method of the determination of pulmonary leak along with the measurement of extravascular lung water by the indicator dilution technique has been used in all the animal experiments in this laboratory where such determinations were necessary for the assessment of therapeutic intervention in experimental ARDS.

RATIONALE

Role of Inflammatory Mediators in ARDS

It is clear that the lung injury in ARDS is mediated by a large number of substances. That some of these substances are inter-related and share a common final pathway or common enzyme systems is becoming more obvious. What

is not clear are the exact inter-relations between these mediators. It is likely that therapeutic intervention with a specific or a combination of specific agents will attenuate the lungs' response to injury and thereby minimize its consequences.

Central to the lungs' response to injury in endotoxin induced ARDS are the neutrophils (12-14). These are not the only culprits but are probably the most easily studied. Sequestration of neutrophils takes place soon after endotoxin infusion in vivo. The exact method of neutrophil aggregation is not known but it is hypothesized that substances such as complement C3a and C5a, leukotrienes and various other chemotactic substances are involved in the initiation of the process (15). In vitro, plasma activated with zymosan causes neutrophil aggregation. Neutrophils become "activated" when they come in contact with a soluble or phagocytic stimulus and manifest this activation as a respiratory burst with an increase in oxygen consumption, activation of the hexose monophosphate shunt and generation of reactive oxygen species and their metabolic products. These products are injurious to cell membranes as well as deactivating enzymes and causing mutagenesis by their action on DNA.

As well as oxygen-free radicals, the neutrophils, platelets, monocytes and lymphocytes can release a number of other factors which have an affect on pulmonary hemodynamics and directly on the endothelium. The products of arachadonic acid metabolism produced by the circulating elements in the blood are thought to cause the acute pulmonary hypertension seen immediately after endotoxin infusion as well as increased lung lymph flow (16-18). Products of the cyclooxygenase system of the arachadonic acid metabolism are thought to cause these effects since increased plasma levels of TxB_2 and 6 Keto $\text{PGF}_{1\alpha}$ are temporally related to the initial pulmonary hypertension and increased lung lymph flow. It has been shown by previous experiments in this laboratory

that these effects can be blunted by a combination of an anti-prostaglandin in conjunction with histamine receptor blockers (19).

The second phase of the lungs' response to endotoxin is characterized by sustained but lower elevations in pulmonary artery pressures and increased protein rich lymph flow secondary to increased capillary permeability. The technique of gamma scintigraphic analysis of radiolabeled tracer across the alveolar capillary membrane developed in this laboratory has been invaluable in assessment of the degree of pulmonary leak in experimental and clinical ARDS and in the response to therapeutic intervention.

This late phase of pulmonary hypertension and protein leak is thought not to be due to the metabolites from the arachadonic cascade and does not respond to inhibitors of this system. It is likely that oxygen-free radicals generated by neutrophils are responsible for a large part of the vascular endothelial cell damage which results in increased permeability (20).

Oxygen-free radicals produced by neutrophils are normally converted to non-injurious substances by the enzymes superoxide dismutase and catalase. However, when these mechanisms are overcome by overwhelming oxygen radical production, intermediates such as H_2O_2 and OH ions occur. These are injurious to cells. The hydroxyl radical particularly causes per-oxidation of lipid membranes and in the presence of transition metals such as copper and iron these membranes disintegrate, lose their integrity and result in increased permeability (21).

Another seemingly potent mediator in the lung injury seen with endotoxin induced ARDS is the inappropriately named platelet activating factor (PAF). This phospholipid which is secreted by many mammalian cells and tissues has been identified as 1-O-alkyl-2-acetyl-sn-glycero-3 phosphorylcholine. PAF aggregates and stimulates both leukocytes and platelets and contracts pulmonary artery and airway smooth muscle in many species (22,23).

EXPERIMENTAL METHODS

1. The Model

The porcine model was used in all the ensuing experiments. Young swine weighing between 14-30 kgs were anesthetized with intramuscular ketamine hydrochloride 25 mg/kg and placed supine. The animals were then given intravenous sodium pentobarbital (10 mg/kg). Following intubation with a cuffed endotracheal tube, they were paralyzed with continuous intravenous pancuronium bromide (0.2 mg/min) to permit mechanical ventilation with 0.5 FiO₂, 5 cm H₂O positive end expiratory (PEEP) and 20 cc/kg tidal volume at a rate which produced a PaCO₂ of approximately 40 torr at the beginning of the experiment.

Catheters were inserted into the left common carotid artery for monitoring systemic arterial blood pressure (SAP) and arterial blood gases, and into the right and left external jugular veins for infusion of *Pseudomonas* (Ps), ^{99m}technetium-labeled serum albumin (TcHSA) and the therapeutic agents to be studied. A thermodilution Swan-Ganz catheter was passed through the right jugular vein into the pulmonary capillary and wedged in position with the balloon inflated. This was used to monitor pulmonary artery pressure (PAP), pulmonary wedge pressure (PWP), and thermodilution cardiac output. Cardiac output was converted to cardiac index (CI) by the formula:

$$CI = \frac{CO}{0.112 BW^{2/3}}$$

where BW is the body weight in kg. Blood gases were measured with a blood gas analyzer (Instrumentation Laboratories, Model 113).

A 5 French femoral artery lung water catheter (American Edwards Laboratories, Model 96-020-5F) was passed into the lower abdominal aorta for measurement of thermal cardiogreen extravascular lung water (EVLW). In this technique 10 ml of iced, green dye solution (2 mg indocyanine green dye in 10 ml 5% dextrose) were injected as a bolus through the proximal port of the Swan-Ganz

catheter as blood was simultaneously withdrawn through the thermistor-tipped femoral artery catheter and a densitometer cuvette (Waters Instruments Inc., Model 402A) linked to a lung water computer (American Edwards Laboratories, Model 9310). The computer measured the mean transit times of the intravascular dye (MTD) and freely diffusable thermal component MIT as well as the cardiac output (CO). EVLW was calculated by the formula:

$$\text{EVLW} = \frac{\text{CO (MTD-MIT)}}{\text{BW (kg)}}$$

Computerized gamma scintigraphy as described above was used as an indicator of pulmonary capillary permeability through the measurement of pulmonary transcapillary albumin flux. Lung-heart radioactivity ratios were constructed with a VAX 8600 computer, and the slope index was calculated by least squares linear regression analysis.

ARDS was induced in the porcine model by infusion of live Pseudomonas aeruginosa, PAO strain, 5×10^8 CFU/ml at 0.3 ml/20kg/min. This infusion was continuous throughout the experiment. In Ps control animals this has been shown to produce a marked physiological deterioration, representative of an acute ARDS, resulting in an immediate significant increase in PAP which persists throughout the entire duration of the experiment. SAP shows a progressive decline as does CI and PaO_2 . EVLW and SI become significantly elevated when compared to saline controls.

We have already established in a previous study that treatment with a combination of cimetidine 150 mg, diphenhydramine 10 mg/kg and ibuprofen 12.5 mg/kg (CID) given intravenously at 20 and 120 minutes after continuous pseudomonas infusion significantly improves all the physiological and hemodynamic parameters in the early stages of lung injury in the model with significant improvement in the hypoxemia, early pulmonary hypertension and pulmonary microvascular injury seen in Ps controls.

2. Delayed Treatment Studies

This study was designed to assess the influence of delayed therapy on survival and physiological parameters in the model. Two groups were studied. Ps control groups were given 5×10^8 CFU/ml as 0.3 ml/20 kg/min (n=5). Treatment group (n=6) received intravenous cimetidine 150 mg/1 hr (C), ibuprofen 12.5 mg/kg/2 hr (I) and diphenhydramine 10 mg/kg continuous infusion (D) given 90 minutes after continuous pseudomonas infusion. Systemic (SAP) and pulmonary artery pressures (PAP), cardiac index (CI), PaO_2 and extravascular lung water (EVLW) were measured every 30 minutes until death. All animals had scintigraphically determined pulmonary albumin flux measured as slope index.

3. CID and Heparin Studies

It was postulated that the late pulmonary hypertensive phase of pseudomonas-induced lung injury was due in part to intravascular coagulation. We have already established that CID attenuates the early phase of the injury and has beneficial effects on the later phase changes. It would therefore, in theory, seem appropriate to administer an anticoagulant to inhibit any intravascular coagulation taking place. Five pigs were studied with heparin administered as a bolus intravenously in a dose of 500 μ /kg at 20 and 120 minutes in combination with CID as described in previous studies.

4. Renal Studies

Cyclooxygenase inhibitors are known to decrease urine output in moderate to large doses due to their action on renal prostaglandins which play a major role in the regulation of renal blood flow (24,25). It had been observed in previous studies in this laboratory that Ps pigs administered ibuprofen had a significantly lower urine output over Ps controls during the course of the studies. It was postulated that cimetidine and diphenhydramine might improve this parameter. PAH, a substance which is secreted by the renal tubules but

neither filtered nor absorbed, as a measure of renal plasma flow, and inulin which is completely filtered as a measure of glomerular filtration, were studied in the pseudomonas porcine model. Six groups of animals were studied. Control (n=5), ibuprofen (I) (n=5) and CID (n=5) alone, Pseudomonas alone (Ps n=4), ibuprofen and Pseudomonas (I + Ps n=5), CID plus Pseudomonas (CID + Ps n=6). Cardiopulmonary parameters and renal function tests (inulin + PAH clearances, BUN and creatinine) were measured at baseline and hourly for three hours thereafter. Blood was collected pre-Pseudomonas and at hourly interval for estimation of blood gases and thromboxane (TxB_2) and 6 keto $PGF_{1\alpha}$ levels. Results obtained from cardiopulmonary measurements substantiated previously published data from this laboratory. The results of the renal function tests are still pending at this time but from initial observations it would appear that the CID + Ps group showed improved renal function over the I + Ps group. Radioimmune assay of the prostaglandin metabolites TxB_2 and 6 keto $PGF_{1\alpha}$ levels are at present ongoing.

5. Anti-platelet Activating Factor Studies

Platelet activating factor (PAF) is a lipid derived from the phospholipid component of cell membranes and is released from a variety of cells including basophils, platelets, neutrophils, mast cells, endothelial cells and macrophages. It contributes to neutrophil margination and tissue edema formation. Administration of PAF in unanesthetized sheep resulted in pulmonary vasoconstriction and an increase in pulmonary lymph flow. A series of studies in the pseudomonas porcine model utilizing an experimental anti-PAF substance, SRI 63-675, were carried out.

6. Studies with Ranitidine

Disturbing reports with the utilization of long-term cimetidine in patients in intensive care units have been documented over the past few years

(26,27). Mental status alterations have been observed with the drug and these are dose and time related. Impairment of hepatic and renal function greatly augment these changes. Mental deterioration has been shown to clear within 24 to 36 hours after stopping the drug. Because of these implications in ARDS patients who may already have impairment of renal and hepatic function, an alternative H_2 receptor blocker, ranitidine which has no such side effects, was assessed in the experimental ARDS model. It has been postulated that in addition to its other actions, cimetidine functions as an oxygen-free radical scavenger and that this action might also be contributory in ARDS treatment with CID. Ranitidine has no such action. A series of studies were conducted using ranitidine in place of cimetidine in combination with diphenhydramine and ibuprofen.

7. Leukocyte Studies

The accumulation of leukocytes with the subsequent release of their toxic products is thought to play an important role in the pathogenesis of the pulmonary microvascular injury seen in septic ARDS. The purpose of this study was to evaluate the behavior of ^{111}In -tagged leukocytes using gamma scintigraphy in the porcine pseudomonas model.

On the day prior to study, 50 cc of whole blood was removed from the anesthetized pig through a central venous catheter. Leukocytes were isolated and labeled with 700 μCi ^{111}In using a standard kit and returned to the animal. On the following day animals were studied according to the standard protocol with the addition of pre-Pseudomonas baseline scintigraphy. Though the animals developed a characteristic ARDS following pseudomonas infusion leukocyte scintigraphy was unable to demonstrate an increase in the slope index. It was thought that the amount of tag that became free prior to study significantly reduced the resolution capabilities of the gamma camera. Evaluation of cell

kinetics by determining counts per minute with whole blood scintillation corrected for free label by subtracting plasma activity. These results indicated that there was rapid mobilization of stored labeled leukocytes immediately following the onset of pseudomonas infusion. However, only three subjects were studied with this technique prior to the abandonment of this inquiry.

8. Sublethal Dose Pseudomonas Model

For projected studies of oxygen radical generation and the use of oxygen radical scavengers in the model, it was determined that a less acute model of ARDS was necessary. Consequently, the previously used dose of Pseudomonas aeruginosa PAO strain which had been infused throughout the entire experiment was limited to a one hour infusion only. This caused a clinical ARDS as measured by hemodynamic parameters with a significant increase in slope index and increase in extravascular lung water concomitant with an increase in permeability. Studies of oxygen radical generation and the effects of oxygen radicals on lipid membranes, as measured by conjugated dienes in vivo, are at present ongoing. It is hoped that measurements of conjugated dienes as an indication of oxygen free radical activity will enable us to study the effects of oxygen-free radical scavengers in the model and thereby elucidate another important mediator in the lung injury seen in ARDS.

RESULTS

1. Delayed Treatment Studies

Two groups were studied: continuous pseudomonas infusion until death (Ps n=5), and continuous Pseudomonas with CID therapy given at 90 and 150 minutes after the onset of infusion (delayed CID, n=6). Results showed that continuous pseudomonas infusion produced a mean survival time of 199 ± 34 minutes while

the delayed administration of CID resulted in a significantly ($p < 0.05$) increased survival time of 328 ± 48 minutes as well as a delayed onset of increased EVLW and cardiovascular collapse (Table 1).

2. CID and Heparin Studies

Five pigs were studied using the therapeutic intervention of CID plus heparin (500 units/kg/min) at 20 and 120 minutes post-Pseudomonas (CIDH). Results (Table 2) indicated that CIDH was essentially indistinguishable from CID alone. Platelet counts done on two CIDH pigs showed a decline from $511,500 \pm 53,500$ at baseline to $224,400 \pm 14,500$ at 180 minutes.

3. Anti-PAF SRI 63-675

Five pigs were studied using an experimental platelet activating factor (PAF) receptor blocker (Sandoz SRI 63-675) given at 20 and 120 minutes post-Pseudomonas. Results showed an attenuation of the early pulmonary hypertension without significant alteration of the other parameters when compared to Pseudomonas alone (Table 3). Further study of the PAF receptor blockers has presently been postponed due to the presence of marked hemolysis following administration. It is felt that this problem must be resolved before further study can occur.

4. RID

Six pigs were studied using the therapeutic combination of ibuprofen 125 mg/kg, diphenhydramine 10 mg/kg and ranidine 25 mg/kg (RID) given i.v. at 20 and 120 minutes after the onset of pseudomonas infusion. RID therapy maintained SI, EVLW and PaO_2 at control levels while PAP progressively rose becoming significantly above control at 90 minutes (Table 2). When compared with CID therapy, RID was found to be equally efficacious although the late pulmonary hypertension was manifested earlier with RID.

DISCUSSION AND CONCLUSIONS

It is evident that the lung injury seen in ARDS is mediated by a number of different substances, some of which may act through the same final pathway. Having established a satisfactory animal model of the syndrome and a reproducible, sensitive and specific method of establishing alveolar capillary damage, all of the experiments over the past year have been involved with therapeutic intervention in the syndrome.

We have established that CID significantly alters the initial pulmonary hypertensive phase in the syndrome and also affects the late pulmonary hypertensive-increased permeability phase to some extent. We have also established that delayed treatment with CID significantly improved survival time in our animal model. We have lately established, in view of the documented side effects of cimetidine, that ranitidine, an alternative H_2 receptor blocker is as efficacious as cimetidine in combination with ibuprofen and diphenhydramine in attenuating all phases of the syndrome.

Initial reports on the beneficial effects of cimetidine and diphenhydramine on ibuprofen-induced renal dysfunction are encouraging. This could be very significant when clinical trials are undertaken with polypharmacy in patients with the syndrome who might already have compromised renal function.

The use of CID in combination with heparin has been shown to be no different than CID alone in this model. The use of large doses of heparin may be associated with considerable morbidity and mortality and it was important to establish that it was of no beneficial effect.

Various anti-platelet activating factor antagonists are, at present, undergoing study. There is no doubt that PAF does play a part as a mediator in ARDS, but how large a part is not yet known. Studies with SRI 63-675 in the porcine pseudomonas ARDS model show significant attenuation of the early

phase of pulmonary hypertension but the antagonist caused significant hemolysis. Further analysis of the drug using a lipid buffer to limit the hemolyzing effect is anticipated.

Studies of neutrophil produced toxic oxygen species are at present ongoing. Major developments in our understanding of the mechanism of action of these free radicals have been made in the past few years and it is suggested that they may play a major part in the lung injury as a result of alveolar capillary membrane damage in ARDS. Direct measurements of these free radicals are not possible in vivo, but measurement of their action on lipid membranes, as reflected by increased levels of conjugated dienes in blood, are possible. Techniques for these measurements are being established and the effects of radical scavengers can then be assessed.

ARDS both in civilian and military patients carries a considerable mortality. The syndrome often occurs in previously fit patients who undergo certain direct pulmonary and non-pulmonary injuries. It has been established that the lung injury seen in ARDS is due to many inflammatory mediators. Elucidation of these mediators and pathways are therefore likely to be necessary for adequate pharmacological intervention and treatment in the syndrome. We have established in animal models that a combination of H_1 and H_2 blockers and a cyclooxygenase inhibitor significantly improve all parameters but do not completely abolish the syndrome. Continued efforts at elucidation and identification of the various inflammatory mediators involved in the syndrome and their blockade by pharmacological intervention will be the purpose of further research in this laboratory. Application of these findings with treatment of septic patients will be undertaken in multicenter trials in the near future.

Gamma scintigraphy as a measure of pulmonary capillary membrane permeability has proven reliable and reproducible both in animal and clinical ARDS and may well become the accepted method for diagnosis of pulmonary leak in the syndrome and for the determination of the efficacy of various pharmacological manipulations.

Table 1
Effect of Delayed CID on Survival in a Porcine Pseudomonas ARDS Model

| Time (Min) | SAP | | CI | | EVLW | |
|------------|----------|----------|-------------|------------------------|-------------------------|-------------------------|
| | PS | CID | PS | CID | PS | CID |
| 0 | 101 ± 15 | 105 ± 20 | 4.0 ± 1.3 | 3.9 ± 0.6 | 4.8 ± 2.1 | 6.8 ± 3.1 |
| 150 | 74 ± 13* | 113 ± 20 | 1.9 ± 0.6** | 2.8 ± 0.6 [†] | 11.4 ± 5.0 [†] | 9.2 ± 5.1 |
| 300 | | 97 ± 56 | | 1.3 ± 0.5 [†] | | 13.3 ± 3.7 [†] |

*p<0.01 versus CID

**p<0.5 versus CID

[†]p<.05 versus 0 min

mean ± SEM

Table 2
Comparison of CID, RID, CID and Heparin with Saline
Controls and Pseudomonas Alone

| <u>PAP</u> | <u>0</u> | <u>90</u> | <u>180</u> |
|------------------------|-----------|-------------|-------------|
| Control | 15 ± 2 | 14 ± 2 | 13 ± 2 |
| Pseudomonas | 15 ± 1 | 38 ± 2 | 35 ± 1 |
| RID | 19 ± 2 | 31 ± 3* | 33 ± 2* |
| CIDH | 20 ± 3 | 24 ± 2** | 32 ± 5* |
| CID | 15 ± 2 | 22 ± 4** | 25 ± 5* |
| <u>PaO₂</u> | | | |
| Control | 173 ± 9 | 201 ± 12 | 203 ± 15 |
| Pseudomonas | 223 ± 15 | 139 ± 26 | 93 ± 17 |
| RID | 213 ± 10 | 226 ± 17** | 218 ± 22** |
| CIDH | 238 ± 8 | 231 ± 18** | 241 ± 11** |
| CID | 232 ± 8 | 240 ± 6** | 208 ± 20** |
| <u>EVLW</u> | | | |
| Control | 4.1 ± 0.9 | 3.7 ± 1.2 | 4.3 ± 1.2 |
| Pseudomonas | 6.8 ± 0.6 | 10.1 ± 1.5 | 14.4 ± 2.2 |
| RID | 4.8 ± 0.9 | 4.6 ± 0.4** | 7.2 ± 1.1** |
| CIDH | 7.1 ± 0.5 | 4.7 ± 0.9** | 7.6 ± 0.6** |
| CID | 6.3 ± 0.4 | 8.7 ± 2.9 | 7.7 ± 0.3** |

*Treatment versus control (p<0.05)

**Treatment versus Pseudomonas (p<0.05)

Table 3
Effects of SRI 63-675 (An Anti-PAF Agent)

| | <u>0</u> | <u>90</u> | <u>180</u> | <u>240</u> |
|------------------------|-----------|------------|------------|------------|
| <u>PAP</u> | | | | |
| Control | 15 ± 2 | 14 ± 2 | 13 ± 2 | |
| Pseudomonas | 15 ± 1 | 38 ± 2 | 35 ± 1 | |
| Anti-PAF | 20 ± 2 | 30 ± 6 | 39 ± 7 | 39 ± 8 |
| <u>PaO₂</u> | | | | |
| Control | 173 ± 9 | 201 ± 12 | 203 ± 15 | |
| Pseudomonas | 223 ± 15 | 139 ± 26 | 93 ± 17 | |
| Anti-PAF | 230 ± 20 | 253 ± 6 | 152 ± 48 | 142 ± 37 |
| <u>EVLW</u> | | | | |
| Control | 4.1 ± 0.9 | 3.7 ± 1.2 | 4.3 ± 1.2 | |
| Pseudomonas | 6.8 ± 0.6 | 10.1 ± 1.5 | 14.4 ± 2.2 | |
| Anti-PAF | 6.6 ± 1.3 | 7.6 ± 0.7 | 10.4 ± 0.7 | 11.6 ± 2.5 |

Insufficient N to perform statistical tests.

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